ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

Comparative Study of Three Spectrophotometric Methods for Diclofenac Quantification in Urine Samples and Pharmaceutical Formulations

Farid Abu Shammala
Assistant Professor, Faculty of Pharmacy, University of Palestine , El-Zahra City, Gaza, PALESTINE
drfaridshammala@hotmail.com

Abstract
This paper reports a comparative study between three spectrophotometric methods for determining the concentration of diclofenac (DCF) in urine samples and pharmaceutical formulations. The first method is based on the reaction between diclofenac and tetrachloro-p-benzoquinone (p-chloranil), in methanol medium, measuring absorbance at 535 nm. The second method based on the oxidation of drugs by Fe(III) in the presence of o-phenanthroline, the formation of tris(o-phenanthroline) iron(II) complex (ferroin) measuring absorbance at 510 nm. The third method is based on the reaction of diclofenac (DCF) with potassium permanganate in an alkaline medium, measuring absorbance at 445 nm. For the first method Beer's law is valid within a concentration range of 1.0–32μgmL^{-1} for diclofenac sodium. The optimum concentration ranges is 2.0–30 for diclofenac sodium. The molar absorptivities are 1.65 X 10^{4} L mol^{-1} cm^{-1}. The limit of detection (LOD) was 1.15x10^{-5} mol l^{-1} and the limit of quantification (LOQ) was 2.5x10^{-5} mol l^{-1} . The method gave a mean percentage recoveries 99.8±1.2% for diclofenac sodium. For the second method Beer's law is obeyed in a concentration range from of 1.5–35μgmL^{-1} with a correlation coefficient of 0.9993 and molar absorptivity of 0.50 x10^{3} l mol^{-1} cm^{-1}. The limit of detection (LOD) was 1.35x10^{-5} mol l^{-1} and the limit of quantification (LOQ) was 4.49x10^{-5} mol l^{-1}. The method gave a mean percentage recoveries 99.2±1.5% for diclofenac sodium. For the third method, under optimal conditions, Beer's law is valid within an optimum concentration ranges of 5-50μgmL^{-1} for diclofenac sodium. The optimium concentration range is 10.0 to 100μgmL^{-1}, with a detection limit of 2.15x10^{-5} mol l^{-1} and the limit of quantification (LOQ) was 5.69x10^{-5} mol l^{-1}. The method gave a mean percentage recoveries 99.5±2.3% for diclofenac sodium. Thus, first method is most accurate method determination of total diclofenac (DCF) in urine samples and pharmaceutical formulations.

Introduction

Diclofenac, 2-[(2,6-dichlorophenyl)amino] benzene acetate (Figure 1), is a synthetic non steroidal compound that is more usually found as sodium or potassium salt. It is used for the treatment of rheumatoid arthritis, ankyllosing spondylitis, osteoarthritis and sport injuries [1]. Its exact mechanism of action remains unknown, but many of its main properties appear to be associated with the inhibition of prostaglandin synthesis [2]. The pharmacological effects of this drug are thought to be related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory process [3]. It is employed mainly in oral formulations, and the some extent, also for intramuscular injection and topical formulation for topical formulations, one of the most wildly used is Voltaren® emulgel, Diclogel® and Dosanac® emulsion gel. The advantage for this type of dosage form found to be effective for the treatment of local inflammatory [4].

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

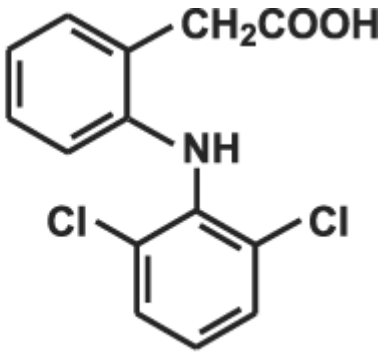

Figure 1. Structural formula of diclofenac.

Various spectrophotometric [5-14], chromatographic [15-21], colorimetric [22-23] and flourimetric [24-27] methods have been developed for quantification of diclofenac sodium. Extensive literature survey revealed that a very few UV method is however reported for the estimation of diclofenac sodium in tablet dosage form. Available UV-Visible spectroscopic method are accurate, precise and reproducible, but has used either costly or unstable solvents. That's why it was thought to make a comparative study between three spectrophotometric methods for determining the concentration of diclofenac (DCF) in urine samples and pharmaceutical formulations. Spectrophotometric methods afford a number of economic advantages over chromatographic techniques while their sensitivity, precision and accuracy are similar.

The present study describes a comparative study of three spectrophotometric method for the determination of diclofenac sodium. The first method is based on the reaction between diclofenac and tetrachloro-p-benzoquinone (p-chloranil), in methanol medium. This reaction was accelerated by irradiating of reactional mixture with microwave energy (1100 W) during 30 seconds, producing a charge transfer complex with a maximum absorption at 535 nm. The second method based on the oxidation of drugs by Fe(III) in the presence of o-phenanthroline. The formation of tris(o-phenanthroline) iron(II) complex (ferroin) upon the reaction of diclofenac sodium or piroxicam with an iron(III)-o-phenanthroline mixture in optimum reaction conditions was measured at 510 nm against a reagent blank prepared in the same manner. The third method is based the oxidation of the drug by potassium permanganate in an alkaline medium and the measurement of the green product formed at 445 nm.

2. Experimental

2.1. Instruments

All the spectrophotometric experiments were carried out by Lambda 2 UV/visible spectrometer of Perkin-Elmer. Julabo HC5 water bath Model, GMBH D-7633 Germany, fitted with test tube netted tray held in vertical position was used for controlling temperature.
- A domestic microwave oven, Panasonic 1100 Watts, Model Junior Plus Intelligent Chaos, was used for heating. The distribution of radiation in the oven cavity was performed similarly to a literature procedure [28, 29]. a "low" power output setting on the microwave for 27 seconds were used, was sufficient for the complete color development. without boiling

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

start. Avoid "high" power output setting on the microwave which will result in boiling and loss of the solution.
- Labtech water bath mantacture of lab instruments .
- Denver sensitive balance instrument ISO 9001.

2.2. Materials, reagents and solutions

All reagents for the three methods were of analytical reagent grade. For the preparation of the solutions and samples, deionised water and grade "A" glassware were used throughout. The solvents used were dioxane (p.a. grade) and methanol (Merck,analytical grade) from (Sigma, St.Louis, USA) was used to prepare a $2.0x10^{-2}$ mol L^{-1} solution in dioxane. Sodium diclofenac was purchased from Sigma, St. Louis, USA. A $5.0x10^{-3}$ mol L^{-1} stock solution of sodium diclofenac was prepared in methanol. The pharmaceutical preparations were purchased locally or directly from the manufacturers and all were tested prior to the listed expiration date. Five commercial brands of injection solutions and three commercial brands of tablets containing diclofenac sodium or potassium were analyzed. The injection samples were package labeled to contain 35 mg of diclofenac per milliliter of solution and the tablets, capsule or suppository samples were 50 mg diclofenac per one sample.

Reagents for the second method: o-Phenanthroline–iron(III) mixture. A 0.50 g amount of o-phenanthroline monohydrate (Fluka, puriss. P.a.), 5.0mL of 1.0M HCl, and 0.40 g of ammonium iron(III) sulphate dodecahydrate (Merck, analytical-reagent grade) were dissolved in and diluted to 250mL with distilled water. The solution is stable for at least 4 weeks if it is stored in a dark, cool location, e.g., in a refrigerator at 3–48C. Stock reference solutions (250 mg/mL) were freshly prepared from pure

samples of diclofenac sodium by dissolving 0.025 g in 100mL ethanol. Acetate buffer solutions of pH values 3.4–5.6 were prepared as recommended previously [29].

For the third method: All solutions were prepared by dissolving the corresponding analytical grade reagent in filtered, distilled water; these solutions were used without further purification.A stock solution (1000 mg L^{-1}) of diclofenac sodium was prepared weekly by dissolving the salt in water. Standard solutions ranging from 1 to 100 mg·l-1 were prepared daily by dilution of this stock solution. Potassium permanganate ($1.0x10^{-3}$ mol L^{-1}) was used as an oxidising agent and NaOH (1.0 mol L^{-1}) as the carrier solution [30, 31].

2.3. General Procedure and Analytical Curve

For the first method : Aliquots (0.5 to 4.0 ml) of a stock standard solution of sodium diclofenac (5.00×10^{-3} mol L^{-1}) were transferred into a 50 ml beakers in order to obtain the analytical curve from 1.25×10^{-4} mol L^{-1} to 2.00×10^{-3} mol L^{-1}. Then, 2.00ml of 2.00×10^{-2} mol L^{-1} p-chloranil solution was added followed by addition of methanol to a final volume of 7 ml. So, the proportion of methanol:dioxane (4:1) was kept constant in all solutions. This procedure was carefully carried out in order to provide an equal heating rate. The beakers were covered with a glass cover, positioned on the center of the oven cavity and heated in the microwave for 30 seconds, at "low" power output (1100 W). Afterwards, the beakers were cooled at room temperature. The working solutions were prepared transferring quantitatively the content inside the beaker to a 10 ml volumetric flask and completing to volume with methanol. Finally, the absorbance was

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

measured at 535 nm (b=1cm) against corresponding reagent blank. The analytical curve was obtained by plotting absorbance against diclofenac concentration and the corresponding linear least square equation was used to convert absorbance into diclofenac concentration, for all analyzed samples.

General Procedure and Calibration for the second method: Into a 25mL calibrated flasks, were pipetted in the order 15.0mL of acetate buffer solution (pH 4.4 and 4.8 for diclofenac sodium and piroxicam, respectively, as optimum pH value), 0.1–3.2mL aliquots of the experimental drug solution, and 3.0mL of o-phen-iron(III) mixture, followed by dilution to volume with water. The flasks were stopped, mixed well by shaking and kept for 15 min in a water bath at, then immediately cooled to room temperature using a cold water bath. The absorbance of the solution was measured at 510nm against a blank solution which has been treated similarly.

2.4. Assay of diclofenac sodium and pharmaceutical preparations

For the first method: From of the stock standard solution of diclofenac sodium ($5.0x10^{-3}$ mol L^{-1}) suitable aliquot for obtain a final diclofenac concentration of $1.0x10^{-3}$ mol L^{-1} was transferred to a 50 ml beaker. Then 2 ml of $2.0x10^{-3}$ mol L^{-1} p-chloranil solution was added. For the pharmaceutical preparations in the first method: Ten tablets were placed in a mortar and ground to the fine powder. An amount of the powder equivalent to 50 mg of pure diclofenac was accurately weighed and dissolved in a minimum volume of methanol. The solution was stirred for 15 minutes and then made up 50 ml with methanol. The solution was filtered in an appropriate apparatus and an aliquot from this filtered solution was analyzed using the procedure previously mentioned.

Procedure for Ampoules: An accurate volume of the solution contained in the ampoules, nominally equivalent to 25 mg of pure diclofenac, was transferred to 25 ml volumetric flask with methanol. Then 4.0 ml of this solution was placed to a 10 ml volumetric flask and analyzed. Procedure for Ampoules for the second method: Into a 100mL calibrated flask, a volume equivalent to 25 mg of diclofenac sodium of the mixed contents of five ampoules was transferred quantitatively and completed to 100mL with ethanol. The assay of the ampoules was completed according to the general procedure. For the third method: Four types of samples were analysed: tablets, ampoules, a topical gel and urine. Tablet powder (0.5 g) was dissolved in an appropriate amount of water in an ultrasonic bath for 3 min; this solution was then diluted with water in a 50.0 ml calibration flask. Suitable aliquots (0.1-0.5 ml) were then diluted 1:20 with distilled water and filtered through a 0.45 µm membrane filter (Millipore). The content of one ampoule was dissolved in 50.0 ml of water in a calibrated flask. Suitable aliquots (0.1-0.5 ml) of this solution were diluted 1:20 with distilled water. A 1.0 g sample of the topical gel was dissolved in 25.0 ml of water and the mixture heated at 40-45°C for 5 min. The aqueous solution was diluted to 100 ml with water in a calibrated flask. Suitable aliquots (0.1-0.5 ml) of this solution were filtered and diluted 1: 2 with distilled water. Urine samples were diluted (1:10) and filtered through a 0.45 µm membrane filter (Millipore).

Procedure for Capsules and Tablets: Into a 100mL calibrated flask an accurately weighted amount of the mixed contents of 20 capsules or tablets, equivalent to 25 mg was

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

transferred quantitatively. The drug was dissolved and diluted to volume with ethanol. The solution was mixed well by shaking and filtered through a suitable filter
paper. The assay of the capsules or tablets was completed according to the general procedure.

Procedure for Suppositories: An accurately weighted amount of the 10 suppositories melted in a small beaker on a water bath with stirring and cool, equivalent to 25mg was transferred quantitatively in a separating funnel. Dissolve in 40mL of hot ethanol and after cooling, extract the fatty with 10mL of ether. Separate the etherial layer and

wash it twice with 10mL of ethanol. Combine the ethanolic extracts and washings
into a 100mL calibrated flask and complete to volume with ethanol. The assay of the suppositories was completed according to the general procedure.

3. Results and Discussion

The first method: The Investigations were performed to establish the most favorable solvent for the formation of the colored product complex for the first method. It is mentioned in the literature that polarity of the solvent used in the reaction between p-acceptors with n-donors can influence on the formation of charge transfer complexes [32]. Therefore, ethanol, methanol, propanol and isopropanol were studied to establish the best solvent for the complex. Results revealed that all the solvents used was found to yield a violet product with the addition of p-chloranil to Diclofenac. Indeed, methanol was found to gave faster color, maximum complex intensity and stability.

The second method: The determination of diclofenac (DCF) drug by Fe(III) in the presence of o-phenanthroline, the formation of tris(o-phenanthroline) iron(II) complex (ferroin) measuring absorbance at 510 nm. Although diclofenac sodium is relatively stable in both light and air, it is easily oxidized in the presence of an oxidizing agents systems such as:

$$Fe^{3+} + e^{-} \leftrightarrows Fe^{2+}$$

In the first method Iron(III)-o-phenanthroline reagent was used for the quantitative determination of diclofenac sodium . The method depends on the formation of a colored complex tris(o-phenanthroline)iron(II) chelate upon the oxidation reaction of these drugs with an iron(III)-o-phenanthroline mixture in acetate buffer of pH 4.4-4.8 for diclofenac sodium. The reaction proceeds through reduction of iron(III) to iron(II) and subsequent formation of the intensive orange–red coloration of the ferroin complex. A detailed study of the optimum chemical conditions for the reduction of phen-iron(III) reaction was performed. The results of the effect of pH was investigated in different buffer medium namely, universal, thiel, borate, and acetate buffer solutions of pH ranges 2.0–12.0. The results revealed that the reduction process occurs in slightly acidic medium and the optimum buffer solution was the acetate. The optimum pH for diclofenac sodium was in the range 3.8–5.0, that pH value was selected for further study to diclofenac sodium, since the results are highly concordant at this pH values. The amount of buffer added to 25mL of solution was also investigated and found to be 15mL which gave marginally the highest absorbance values.

The third method: The third method is based on the reaction of diclofenac (DCF) with potassium permanganate (MnO_4^-) in an alkaline medium, measuring absorbance at 445 nm. The results shows that DFC is oxidized by in alkaline media, and the green

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

product formed (manganate ions) shows an absorption maximum at 445 nm. The colour is stable in alkaline solution but disappears in acidic and neutral media.

The results shows water is not an ideal medium for storing DCF since hydrated

DFC species are generated, especially hydroxyl species [33].Table 2 shows the absorbance results obtained for each trial. All experiments were performed in triplicate in order to calculate the residual error; measurements were performed with solutions containing 50.0 mg L^{-1} of DFC held for different storage times to provide three noise factor settings.

The effect of reagent concentration:

For the first method: Five aliquots of each drug solutions were taken from standard stock solution and transferred to 10ml volumetric flask to get a final concentration of 5, 10, 15, 20 and 25 µg/ml of diclofenac and the volume was completed with the distilled water and each flask content was measured to determine the absorbance at all the selected wavelength. For simultaneous equation method the absorbance of all standard solutions were measured at at 535 nm, the calibration curves of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for diclofenac sodium were determined. The influence of the p-chloranil concentration on the formation of the complex was also studied. The concentrations of 5, 10, 15, 20, 25 and 50 µg/ml were used. It was observed an increase in the quantity of the complex formed when the concentration of the p-chloranil goes from 5.0 to 50.0 50 µg/ml , remaining constant in upper concentrations. Therefore the concentration of 50.0 µg/ml was selected, which is in accordance with related results. The results of the effect of reagents concentration has shown that diclofenac need equivalent numbers of mole of p-chloranil for the production of maximum color intensity with reproducible results. Higher concentrations of p-chloranil did not affect the color intensity. For the second method: The results obtained showed that at least 2.5mL solution of the prepared reagent should be present to achieve maximum color development. However, 3.0mL of reagent was used in the present study to insure complete color formation and quantitative reaction at the upper limit of the calibration curves. The color formed under these conditions was stable for more than 12 hr.

Analytical evaluation of the diclofenac complexes

The absorption spectrum of the diclofenac complexes was investigated in the double beam mode against a reagent blank in the range 400–600 nm. Automatic base line correction was employed, while the same base line was determined and checked with both samples and blank cells filled with reagent blank solution. The calibration graph was obtained according to the above general procedure. The linearity (six replicates for seven different concentrations) was checked by a linear least-squares treatment. All the spectral characteristics, the measured or calculated factors , the analytical parameters and the optical characteristics for the spectrophotometric determinations of diclofenac colored complexes by the proposed methods are given in Table1-4.

Linearity and range

For the first method Beer's law is valid within a concentration range of 1.0–32µgmL-1 for diclofenac sodium. When p-Chloranil was evaluated as a chromogenic reagent for spectrophotometric determination of diclofenac. Under the proposed experimental conditions a linear response between

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

absorbance and diclofenac concentration was verified. Beer's law was obeyed in a concentration range from 1.5–35µgmL-1 with correlation coefficient 0.9993. The spectrophotometric method showed a molar absorptivity of 1.65 X 10^4 $Lmol^{-1}cm^{-1}$. , indicating a good sensitivity for the samples analyzed. The method gave a mean percentage recoveries 99.8±1.2% for diclofenac sodium. For the second method Beer's law is obeyed in a concentration range from of 1.5–35µgmL-1 with a correlation coefficient of 0.9993 and molar absorptivity of 0.50 x103 l mol-1 cm-1. The method gave a mean percentage recoveries 99.2±1.5% for diclofenac sodium. Permanganate is well known as the superior oxidizing agent with its high absorptivity, has been found selective in controlled conditions for the assay of some medicines in their formulations [32, 33]. In the third method, it has been found that diclofenac can be oxidized by permanganate in sulphuric acid media. The oxidized form of diclofenac is spectrophotometrically detectable at 450 nm. For the permanganate diclofenac colored complex method, under optimal conditions, the linear range of the calibration curve varied from 10.0 to 100µgmL-1, the method gave a mean percentage recoveries 99.5±2.3% for diclofenac sodium. The regression equations for the described procedures were derived using the least-square method.

Sensitivity, Accuracy, and Precision

The mean Sandell sensitivity as calculated from Beer's law is presented in Table 2-4. In order to determine the accuracy and precision of the method, solutions containing three different concentration of diclofenac sodium 5.0, 10.0, and 25.0µgmL^{-1}, were prepared and analysed in quintuplicate. The measured standard deviation (S.D.), relative standard deviation (R.S.D.), the standard analytical error and confidence limits, (Table 1) can be considered satisfactory, at least for the level of concentrations examined. The relative standard deviation (%RSD) for the first method was found to be in the range of 1.03-1.6 (510 nm) for diclofenac and for the second method in the range of 1.64-1.91 (535 nm) for diclofenac. The %RSD for third method found in the range of 1.92-2.62 (445nm) for diclofenac, respectively as shown in Table 1. Accuracy of the methods was confirmed by doing recovery studies from marketed formulation at three concentration levels of standard addition. The %recoveries found for the first method was 99.9-101.2 for diclofenac. For second method the %recoveries found to be 97.9-99.7 for diclofenac and for the third method the %recoveries found to be 96.2-97.8 for diclofenac, respectively as shown in table 6.

Limit of Detection and Limit of Quantification

The limits of detection (LOD) and quantification (LOQ) were also examined. LOD was obtained as the concentration of a solute resulting in a peak height three times the baseline noise level (3.SDblank/slope of analytical curve). LOQ was obtained as the concentration of solute resulting in a peak height ten times the baseline noise level (10.SDblank/slope of analytical curve). For the tris(o-phenanthroline) iron(II) complex (ferroin) method the limit of detection (LOD) was $1.15x10^{-5}$ mol L^{-1} and the limit of quantification (LOQ) was $2.5x10^{-5}$ mol L^{-1} . For diclofenac and tetrachloro-p-benzoquinone (p-chloranil) colored complex, the limit of detection (LOD) was $1.35x10^{-5}$ mol L^{-1} and the limit of quantification (LOQ) was $4.49x10^{-5}$ mol L^{-1}. For the permanganate diclofenac colored complex method, under optimal conditions, the detection limit was $2.15x10^{-5}$ mol L^{-1} and the limit of quantification (LOQ) was $5.69x10^{-5}$ mol L^{-1}.

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

Table 1. Evaluation of accuracy and precision of the proposed three method.

formulations	weighting (μgmL^{-1})	Found± S.D.	S.D. (%)	Standard error	Confidence Limits .
Method 1	5	5.02±0.081	1.55	0.017	5.02±0.109
	10	10.40+0.067	1.03	0.013	10.50±0.112
	25	25.00+0.119	1.61	0.014	25.20±0.123
Method 2	5	5.80+0.115	1.64	0.022	5.30±0.196
	10	10.00±0.081	1.76	0.017	10.60±0.181
	25	25.40+0.135	1.91	0.021	24.70±0.191
Method 3	5	5.45+0.115	1.92	0.027	5.20±0.281
	10	10.50±0.091	2.62	0.029	10.20±0.291
	25	25.70+0.115	1.98	0.028	25.20±0.171

Table 2-Analytical parameters for the spetrophotometric determination of diclofenac.

Parameters	Method 1
λ_{max} (nm)	535
ϵ ($Lmol^{-1}cm^{-1}$)	1.65×10^4
Sandel sensitivity($ngcm^{-2}$) [a]	2.78
Stability (min)	90min
Beer's Law range (mol L^{-1})	$1.35x10^{-4}$ - $2.5x10^{-3}$
LOD (mol L^{-1})[b]	$1.15x10^{-5}$
LOQ (mol L^{-1})[c]	$2.5x10^{-5}$
Regression line equation: A = mC±z[b] (y)[d]	
m (Slope ± S.D.) (n = 5)	0.036±0.022
z (Intercept± S.D.) (n = 5)	-0.0320±0.044
Correlation coefficient (r)	0.9995
Beer's Law range (μgmL^{-1})	1.0—32
Optimum concentration range (μgmL^{-1})	2.0—30

- a: Average of six determinations (the values obtained are referred to diclofenac sodium
- b: Limit of detection
- c: Limit of quantification
- d: A: absorbance , C: is the concentration (mol L^{-1}) of DFD.

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

Table 3- Analytical parameters for the spetrophotometric determination of diclofenac.

Parameters	Method 2
λ_{max} (nm)	510
ϵ ($Lmol^{-1}cm^{-1}$)	0.50×10^3
Sandel sensitivity($ngcm^{-2}$) [a]	2.78
Stability (min)	120min
Beer's Law range (mol L^{-1})	1.35×10^{-4} - 2.5×10^{-3}
LOD (mol L^{-1})[b]	1.35×10^{-5}
LOQ (mol L^{-1})[c]	4.49×10^{-5}
Regression line equation: A = mC±z[b] (y)[d]	
m (Slope ± S.D.) (n = 5)	0.038±0.002
z (Intercept± S.D.) (n = 5)	-0.033±0.004
Correlation coefficient (r)	0.989±1.5
Beer's Law range (μgmL^{-1})	1.5—35
Optimum concentration range (μgmL^{-1})	2.0—45

- a: Average of six determinations (the values obtained are referred to diclofenac sodium
- b: Limit of detection
- c: Limit of quantification
- d: A: absorbance , C: is the concentration (mol L^{-1}) of DFD.

Table 4- Analytical parameters for the spetrophotometric determination of diclofenac.

Parameters	Method 3
λ_{max} (nm)	445
ϵ ($Lmol^{-1}cm^{-1}$)	1.93×10^3
Sandel sensitivity($ngcm^{-2}$) [a]	2.78
Stability (min)	60min
LOD (mol L^{-1})[b]	1.35×10^{-5}
LOQ (mol L^{-1})[c]	4.49×10^{-5}
Regression line equation: A = mC±z[b] (y)[d]	
m (Slope ± S.D.) (n = 5)	0.0526±0.022
z (Intercept± S.D.) (n = 5)	-0.0270±0.008
Correlation coefficient (r)	0.9995
Beer's Law range (μgmL^{-1})	5-45
Optimum concentration range (μgmL^{-1})	10.0 to 100

- a: Average of six determinations (the values obtained are referred to diclofenac sodium
- b: Limit of detection
- c: Limit of quantification
- d: A: absorbance , C: is the concentration (mol L^{-1}) of DFD.

Repeatability

The method repeatability was determined using six determinations at 100% of the test concentration (0.25 mg/ml), the following results are shown in Table 5. RSD values are given for each DFD. In every case, RSD values were better than 1.5% indicating the repeatability of the proposed method is acceptable.

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

Table 5-Results of repeatability test for the three methods in pharmaceutical raw material and formulations.

		Content (%) [a]		
formulations	weighting (mg)	Method 1	Method 1	Method 1
Tablets	42mg	99.20	98.50	95.25
		100.20	95.43	98.20
		99.60	98.23	96.23
Capsules	35mg	100.80	96.34	98.12
		99.92	92.40	96.33
		100.40	97.45	93.65
Ampoule	32mg	100.25	99.98	96.44
		100.34	97.40	94.23
		99.67	96.30	96.21
Suppository	100mg	100.43	98.15	95.24
		100.42	95.30	97.75
		99.90	97.60	95.45

a: Mean of five determinations.

Table 6- Results of recovery test for the three methods in pharmaceutical raw materials and formulations by the proposed methods.

		Recovery[a] ± S.D. (%)		
formulations	weighting (mg)	Method 1	Method 2	Method 3
Tablets	25mg	99.90±0.091	98.00+0.162	96.50+0.188
		100.40+0.067	100.00+0.173	97.50+0.211
		99.90+0.134	99.20±0.051	97.20±0.043
Capsules	50mg	100.80+0.115	98.70±0.060	97.30±0.091
		101.00±0.081	97.80±0.044	97.60±0.091
		100.40+0.135	98.90±0.034	97.70±0.091
Ampoule	75mg	100.15+0.115	100.20±0.091	97.20±0.091
		99.90±0.091	99.50±0.091	96.20±0.091
		99.90±0.091	99.30±0.091	96.20±0.091
Suppository	100mg	100.80+0.115	97.40±0.091	97.20±0.091
		100.40+0.115	98.20±0.091	96.20±0.091
		99.90±0.091	98.70±0.091	96.20±0.091

a: Mean of five determinations.

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

Recovery from pharmaceutical formulations

In order to evaluate the analytical usefulness of the proposed three methods, it was applied to the determination of studied diclofenac sodium in commercial pharmaceutical formulations by assaying raw materials, capsules, injections and tablets.. The assay showed the DFC content of these products to be within pharmacopeial limits. The results were listed in Table 6 The results showed a good recoveries obtained for the three method with excellent recovery for the first method. The percentage of diclofenac recovery in the estimated formulation for the first method was found to be 99.9-101.2 for diclofenac. For second method the %recoveries found to be 97.9-99.7 for diclofenac and for the third method the %recoveries found to be 96.2-97.8 for diclofenac, respectively as shown in table 6.

Recovery from urine sample

Analysis of urine samples 10 fold of diluted urine or serum sample was processed according to the procedure mentioned in experimental section. For each spectral observation, the diluted blank and DFC containing urine sample of the same individual were processed. In all samples, the absorbance of blank was considered as zero for DFC. A clear signal represented by blue line indicates the presence and absorption spectrum of DFC in the urine sample of the individual. The results also shows that DFC containing urine sample from the individual administered with 50 mg uptake of DFD tablet, capsule, ampoule or suppository has a higher absorbance value as compared to his blank serum sample showing the presence of DFC. The spiking of each diluted urine sample was performed with 40 µl of DFC solution in order to confirm the peak signal and hence the presence of DFC in the urine sample. Table 7 shows the recovery of DFC in a spiked urine sample.

Table 7- Recovery of DFC in a spiked urine sample.

		Found concentration[a] ± S.D. (%)		
Sample	added concentration	Method 1	Method 2	Method 3
Urine	0.0mg	< L.D.	< L.D.	< L.D.
	10mg	10.20+0.115	9.920±0.060	9.80±0.191
	20mg	20.05+0.115	19.92±0.243	18.95±0.091
	50mg	50.18+0.115	50.040.091	48.90±0.228

a: Concentration units are mg L^{-1}. In parenthesis: RSD% for n=5.

The results of DFC in 5 urine samples after the uptake of 50mg DFC by the currently studied methods were observed (Table 8). Each sample of urine with specific number was collected from same individual at different times as described in experimental section. DFC in each sample was determined with the help of linear equation and multiplied by dilution factor 10 in order to get the actual concentration of the ingested DFC transferred to respective urine sample. The data show that the urine contents of DFC have the range of 46.2–62.5 % for a 50 mg ingested tablet, capsule, ampoule or suppository for the first method, the range of 44.2–60.9 % for a 50 mg ingested tablet for the second method, the range of 40.2–52.2 % for a 50 mg ingested tablet for the third method . The remaining DFC may be present in plasma and/ or converted to inactive metabolites. As there is no satisfactory data available for DFC contents in these fluids hence we rely upon the currently investigated data. Further studies in this regard could throw sufficient

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

light upon actual kinetics of this drug and its fate in the body fluids. The RSD of the recovery from the three methods was below 5% for all standard solutions and samples. The results for determining the recovery of DFC form urine sample after 50 mg uptake tablet, capsule, ampoule or suppository are shown in table 8. In order to test the interference from compounds that are usually present in the pharmaceutical sample of DFD, the effect of the constituent pharmaceutical excipients were studied; including sucrose, sorbitol, sodium benzoate, glycerol and calcium phosphate. Solutions containing 20.0 mg L^{-1} of DCF and the foreign compound at higher concentrations (maximum 100:1) were analysed. The interfering concentration of each compound was considered that which caused a variation in the response greater than or equal to ± 5% compared to the response obtained in its absence. The results showed that, at the concentrations in which they were present in the samples tested, none of these excipients interfered in the determination of DCF.

Table 8- Results of recovery test of DFD formulation for the three methods in urine samples after uptake of 50 mg DFD.

		Recovery[a] ± S.D. (%)		
formulations	weighting (mg)	Method 1	Method 2	Method 3
Tablets	50mg	46.20±0.091	56.00+0.162	40.50+0.188
		55.40+0.067	56.00+0.173	47.50+0.211
		54.00+0.119	45.23+0.208	45.20+0.123
Capsules	50mg	55.80+0.115	44.20±0.060	44.30±0.191
		53.00±0.081	55.80±0.144	51.60±0.241
		50.40+0.135	60.90±0.134	40.70±0.251
Ampoule	50mg	62.45+0.115	56.20±0.243	48.20±0.091
		54.70+0.115	57.90±0.151	43.20±0.154
		56.30±0.091	59.30±0.091	48.20±0.248
Suppository	50mg	45.80+0.115	57.40±0.091	45.20±0.228
		43.60±0.091	58.70±0.191	44.20±0.221
		44.20+0.115	47.90±0.091	47.20±0.191

a: Mean of five determinations.

4. Conclusion

Comparative study of three spectrophotometric methods of diclofenac quantification in pharmaceutical formulations and urine samples was made, the results has shown that there was no significant difference between them. The first method is based on the reaction between diclofenac and tetrachloro-p-benzoquinone (p-chloranil), measuring absorbance at 535 nm. The second method based on the oxidation of drugs by Fe(III) in the presence of o-phenanthroline, the formation of tris(o-phenanthroline) iron(II) complex (ferroin) measuring absorbance at 510 nm. The third method is based on the reaction of diclofenac (DCF) with potassium permanganate in an alkaline medium, measuring absorbance at 445 nm. .The three method proposed is simple, sensitive, rapid, low-cost, does not involve any

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

pre-treatment or extraction steps and gives precise and accurate results. In the first method significant improvements in the time of analysis could be attained using the microwave energy (1100 W) for 30 seconds. We have found that all the quantification methods give good results, and the first method is the most accurate, sensitive and reasonably precise one, have low standard deviations (less than 3-4%) and represent straightforward method to determine diclofenac with the highest recovery from both pharmaceutical formulations and urine samples .

References

[1] Sweetman, S C (Ed.), Martindale: The complete drug reference, 35th ed, Pharmaceutical Press, London & Chicago, 2006.
[2] Adeyeye, C M, Li, P K In Analytical Profiles of Drug Substances , Florey, K., editor; Academic Press: New York, 1990, vol. 19, p. 123.
[3] "United State Pharmacopeia"USP 28, 2005, United States harmacopeial Convention, INC. Asian Edition.
[4] Martindale A, The Extrapharmacopeia. The Pharmaceutical Press, London, 29th edition, 1980, 12.
[5] Sastry C P, Prasad T, Suryamarayana M V. Spectrophotometric method for determination of diclofenac sodium in bulk samples and pharmaceutical preparations. Analys1989; 114: 513-516.
[6] Sastry C P, Suryanarayana M V. Visible spectrophotometric determination of diclofenac sodium in bulk samples and pharmaceutical preparations. Microchem. Journ. 1989; 39: 277-282.
[7] Agrawal Y K, Shivramchandra K. Visible spectrophotometric method for determination of diclofenac sodium. Journ. Pharm. Biomed. Anal.1991; 9: 97-100.
[8] Validya R, Parab R S. Validated spectrophotometric method for determination of diclofenac sodium in bulk formulation. Indian Drugs 1995; 32: 194-196.
[9] Perez R T, Martınez L C, Sanz A, Miguel M T S. Spectrophotometric method for drtermination of diclofenac sodium in pharmaceutical preparations. Journ. Pharm. Biomed. Ana.l 1997; 16: 249-254.
[10] Agatonovic K C, Zivanovic L, Zecevic M, Radulovic D. Validated spectrophotometric method for drtermination of diclofenac sodium. Journ. Pharm.Biomed. Anal.1997; 16: 147-153.
[11] Bucci R, Magri A D, Magri A L. Validated spectrophotometric method for drtermination of diclofenac sodium in tablets, suppositories and gel. J. Anal. Chem.1998; 362: 577-582.
[12] El-Didamony M, Amin A S. Spectrophotometric method for determination of diclofenac sodium in pure and pharmaceutica preparations.Anal. Let.t 2004; 37: 1151-1162.
[13] Matin A, Farajzadeh M A, Jouyban A. Spectrophotometric method for determination of diclofenac sodium. Farmaco 2005; 60:855-858.
[14] Kormosh Z O, Hunkaa I P, Bazel Y R. Spectrophotometric method for determination of diclofenac sodium in pharmaceutical preparations. Journ. Chin. Chem. Soc. 2008; 55: 356-361.
[15] Lala L G, Mello P M, Naik S R. Validated hptlc method for determination of diclofenac in serum. Journ. Pharm. Biomed. Anal. 2002; 29: 539-544.
[16] Tehrani M M H, Maryam E. Hplc method for determination of synthetic precursor of diclofenac sodium. Iranian J. Pharm. Res.2002; 1: 51-53.
[17] Shafiee M, Amini M, Hajmahmodi M. Validated hplc and glc method for determination of diclofenac. Sci. Islam. Repub. Ira. 2003; 14: 21-25.
[18] Hanysova L, Mokry R, Kastner P, Klimes J. Rphplc method for determination of diclofena in injectables and gels. Chem. Pap. 2005; 59: 103-108.
[19] Thongchai W, Liawruangrath B, Thongpoon M C T. Validated hptlc method for determination of diclofenac sodium. Chi. Mai. Journ. Sci. 2006; 33: 123-128.
[20] Bertocchi P E, Antoniella L, Valvo S, Alimonti A, Momoli J. Methotrexate determination in pharmaceuticals by enantioselective HPLC, J. Pharm. Biomed. Anal, 37, 679 (2005).
[21] Chmieleska P A, Bieniecki M, Lamparczyk H. Validated rphplc method for determination of diclofenac sodium in human plasma. Biomed. Chromatogr. 2006; 20: 119-124.
[22] Sznitowska M, Stokrocka G M. Hplc method for determination of diclofenac in pharmaceutical formulations. Acta Poloni Pharma Drug Res. 2007; 63: 401-405.

ISSN: 0974-5343
IJMST (2018), 8(2):11-24
DOI: http://doi.org/10.5281/zenodo.1665833

[23] El Sherif Z A, Walash M I, El-Tarras M F, Osman A O. Colorimetric method for determination of diclofenac sodium in standard and pharmaceutical formulation. Anal. Lett. 1997; 30: 1881-1896.
[24] Olakunle S I, Olajire A A, Bolaji A O, Ajibola A O. Colorimetric assay method for diclofenac in tablets. Pak. J. Pharm. Sci. 2006; 19: 134-141.
[25] Garcia M S, Albero M I, Sanchez P C, Molina J. Spectroflourimetric determination of diclofenac sodium in tablets and ointment. Journ. Pharm. Biomed. Anal. 1998; 17: 267-273.
[26] Garcia S, Sanchez P C, Albero I, Garcia F. Flourimetric and spectrophotometric metod for determination of diclofenac in bulk and pharmaceutical samples. Microchim. Acta. 2001; 3: 67-71.
[27] Marcela C, Liliana B. Spectroflourimetric determination of diclofenac sodium. Analy. Sci. 2006; 22: 431-434.
[28] Kingston H M, Walter P J, Chalk S, Lorentzen E, Link D, Kingston H M, Haswell S J (Eds.). Microwave - Enhanced Chemistry - Fundamentals, Sample Preparation and Applications, ACS, Washington, 1997, p. 772.
[29] Morales-Rubio A, Cerezo J, Salvador A. Hydrolysis of Phytic Acid by Microwave Treatment: Application to Phytic Acid Analysis in Pharmaceutical Preparations, De la Guardia M. Microchem. J. 1993; 47 : 270-277.
[30] Sultan S M, Idris A M, Ibrahim K E E. Sequential injection kinetic method for the assay of aspirin in drug formulations. Journal of Flow Injection Analysis. 2005, 22 (2): 118-122.
[31] Idris A M, Sultan S M, Ibrahim K E E, Assubaei F N. Sequential injection spectrophotometric kinetic method for the determination of paracetamol in dosage forms. Journal of Flow Injection Analysis. 2005, 22 (2): 123-128.
[32] Bebawy L I, El-Kousy N, Suddik J K, Shokry M. Spectrophotometric determination of fluoxetine and sertraline using chloranil, 2, 3 dichloro-5, 6 dicyano benzoquinone and iodine. J. Pharm. Biomed. Anal. 1999; 21: 133-138.
[33] Bertocchi P, Antoniella E, Valvo L, Alimonti S, Momoli A. Sequential Injection Spectrophotometric Determination of Diclofenac in Urine and Pharmaceutical Formulations J. Pharm. Biomed. Anal, 2005; 37: 679-285.